Journal of Chromatography, 128 (1976) 300–301

CHROM. 9344

Note

Numerical calculation of the separation parameters in gel chromatography

HUBA KALÁSZ and KÁLMÁN MAGYAR
Semmelweis Medical University, Department of Pharmacology, Üllöi ut 26, 1085 Budapest (Hungary)
and
WILLIAM T. BARNES
Yale University, Department of Internal Medicine, 333 Cedar Street, New Haven, Conn. 06510 (U.S.A.)
(First received June 24th, 1975; revised manuscript received May 11th, 1976)

The rapid development of different chromatographic methods has led to the need for a theoretical background for predicting separation and maximal resolution. The basic equation for resolution (R_s) is

$$R_s = \frac{2\,(t_2 - t_1)}{w_2 + w_1} \tag{1}$$

where t_1 and t_2 are the elution times of peaks 1 and 2 and w_1 and w_2 are the widths of the peaks at the baseline. Eqn. 1 is essential for the evaluation of a given chromatogram, but it is not generally applicable for estimating the probable results of subsequent experiments.

If we substitute k'_2, α and N in eqn. 1, where $\alpha = (t_2 - t_0)/(t_1 - t_0)$, $k'_2 = K_1/K_2$* and $N = 16\,(t_2/w_2)^2$, eqn. 2 is obtained[1,2]:

$$R_s = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_2}{1 - k'_2}\right) N^{\frac{1}{2}} \tag{2}$$

The different terms in eqn. 2 represent selectivity, capacity and efficiency (the symbols are discussed in detail elsewhere[1,2]).

By introducing N_{eff} (effective or reduced plate number), eqn. 2 can be further simplified:

$$R_s = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right) N_{\mathrm{eff}}^{\frac{1}{2}} \tag{3}$$

where $N_{\mathrm{eff}} = 16\,(t_2 - t_1)/w_2$ and t_0 is the elution time of substances eluted at the void volume (V_0). Even if α is known, calculations by eqns. 2 and 3 are not simple.

The use of eqns. 2 and 3 for gel chromatography can cause considerable complications. In gas chromatography and in certain types of liquid chromatography (*e.g.*, high-performance liquid chromatography with ion exchange, partition or ad-

* K_1 and K_2 indicate the distribution of cubstances: K = (total amount of substance in phase x)/(total amount of substance in phase y) (see ref. 1, p. 30).

sorption) the solvent is eluted first, and the position of the substance to be separated must be far enough from the solvent peak and further from V_0 (or t_0). In gel chromatography, however, the substances can be eluted at or near to V_0. This situation exists mainly in preparative gel chromatography, the most desired substance must be eluted first, so that it will not be contaminated with tailing of other peaks that are eluted earlier. In this instance, *e.g.*, when t_1 is identical with or very close to t_0, N_{eff} is meaningless and the value of $(t_2 - t_0)/(t_1 - t_0) = \alpha$ will be difficult to handle, even when t_2/t_1 or $(t_2 - t_1)/t_1$ is in the right range.

For calculation of the optimal cycle number in recycling gel chromatography[3], we propose the use of the relative peak distance, $m = (t_2 - t_1)/t_1$. This value can also be used for the simple calculation of maximal resolution, with the following substitutions:

$$R_s = \frac{2\,(t_2 - t_1)}{w_2 + w_1} \approx \frac{(t_2 - t_1)}{w_1} = \left(\frac{t_2 - t_1}{t_1}\right)\left(\frac{t_1}{w_1}\right) \approx \frac{1}{4}\, m\, N^{\frac{1}{2}}$$

assuming that $w_1 \approx w_2$, $N_2 = 16\,(t_2/w_2)^2 \approx N_1 = 16\,(t_1/w_1)^2$ and $\sqrt{N_2} \approx \sqrt{N_1} = 4\,t_1/w_1 \approx N$.
That is,

$$R_s = \tfrac{1}{4}\, m\, N^{\frac{1}{2}} \qquad (4)$$

Eqn. 4 shows that the resolution is proportional to the relative peak distance and the square root of the theoretical plate number. When one of these three characteristics is chosen, the other two depend on each other.

Eqn. 4 is preferably used in gel chromatography, but it can be applied to any type of chromatography in which the concept of the theoretical plate number[4] is valid.

Eqn. 4 is very easy to use, as shown by the following examples:

(1) when $N = 400$, for resolution $R_s = 1$, the relative peak distance must be at least 0.2 (20%);

(2) if the relative peak distance is 0.04 (4%), then $N = 100$ gives a possible value of $R_s = 0.1$, $N = 1000$ gives $R_s = 0.31$, $N = 10{,}000$ gives $R_s = 1$ and $N = 40{,}000$ gives $R_s = 2.0$.

REFERENCES

1 B. L. Karger, L. R. Snyder and C. G. Horvath (Editors), *An Introduction to Separation Science*, Wiley-Interscience, New York, 1974.
2 J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971.
3 H. Kalász, J. Nagy and J. Knoll, *J. Chromatogr.*, 107 (1975) 35.
4 J. C. Giddings, *Dynamics of Chromatography*, Vol. 1, Marcel Dekker, New York, 1965.